

Claims:

Sub C1 1. A modified pullulanase which is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

5 2. The modified pullulanase of Claim 1 wherein said pullulanase is a modification of a pullulanase obtainable from a gram positive or a gram negative microorganism.

10 *Sub C2* 3. The modified pullulanase of Claim 2 wherein the gram positive microorganism includes *B. subtilis*, *B. deramificans*, *B. stearothermophilus*, *B. naganoensis*, *B. flavocaldarius*, *B. acidopullulyticus*, *Bacillus sp APC-9603*, *B. sectorramus*, *B. cereus*, and *B. fermus*.

15 4. The modified pullulanase of Claim 2 wherein the gram negative microorganism includes *Klebsiella pneumoniae* and *Klebsiella aerogenes*.

20 *Sub C3* 5. The modified pullulanase of Claim 3 wherein the *B. deramificans* pullulanase has the designation T89.117D in the LMG culture collection.

6. The modified pullulanase of Claim 1 wherein the modification is a deletion of amino acids from the amino terminus of about 100 amino acids.

25 7. The modified pullulanase of Claim 1 wherein the modification is a deletion of amino acids from the amino terminus of about 200 amino acids.

8. The modified pullulanase of Claim 1 wherein the modification is a deletion of amino acids from the amino terminus of about 300 amino acids.

30 9. The modified pullulanase of Claim 6 wherein the deletion is 98 amino acids from the amino terminus of *B. deramificans* pullulanase.

10. The modified pullulanase of Claim 6 wherein the deletion is 102 amino acids from the amino terminus of *B. deramificans* pullulanase.

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11. The modified pullulanase of Claim 1 wherein the modification is an addition of at least one amino acid to the amino terminus of the mature pullulanase amino acid sequence.

12. The modified pullulanase of Claim 11 wherein the pullulanase is obtainable from *Bacillus deramificans* and the additional amino acid at the amino terminus is an Alanine.

10 13. Modified pullulanase produced by the method comprising the steps of obtaining a recombinant host cell comprising nucleic acid encoding mature pullulanase, culturing said host cell under conditions suitable for the production of modified pullulanase and optionally recovering the modified pullulanase.

15 14. The modified pullulanase of Claim 13 wherein the nucleic acid encoding mature pullulanase has at least 70% identity to the polynucleotide sequence as shown in SEQ ID NO:1.

20 15. The modified pullulanase of Claim 13 wherein the host cell is *B. licheniformis* which comprises a first gene encoding Carlsberg protease and a second gene encoding endo Glu C protease, the first and/or second gene which codes for the protease(s) having been altered such that the protease activity is essentially eliminated.

25 16. A nucleic acid comprising a polynucleotide sequence encoding a modified pullulanase of Claim 1.

17. The nucleic acid of Claim 16 having at least 70% identity to the polynucleotide sequence shown in SEQ ID NO: 1.

30 18. The nucleic acid of Claim 16 having the polynucleotide sequence as shown in SEQ ID NO:1.

19. An expression vector comprising the nucleic acid of Claim 16.

35 20. A host microorganism comprising the expression vector of Claim 19.

21. The host microorganism of Claim 20 wherein said microorganism is a Bacillus.

22. The host microorganism of Claim 21 wherein said Bacillus includes 5 *B.subtilis*, *B.licheniformis*, *B.lentus*, *B.brevis*, *B.stearothermophilus*, *B.alkalophilus*, *B.amyloliquefaciens*, *B.coagulans*, *B.circulans*, *B.lautus* and *Bacillus thuringiensis*.

23. A method for the production of a modified pullulanase in a host cell comprising the steps of:

- 10 a) obtaining a recombinant host cell comprising nucleic acid encoding a modified pullulanase; and
b) culturing the microorganism under conditions suitable for the production of the modified pullulanase.

15 24. The method of Claim 23 further comprising the step of:
c) recovering the modified pullulanase.

25 25. The method of Claim 23 wherein the host cell is a Bacillus including *B.subtilis*, *B.licheniformis*, *B.lentus*, *B.brevis*, *B.stearothermophilus*, *B.alkalophilus*,
20 *B.amyloliquefaciens*, *B.coagulans*, *B.circulans*, *B.lautus* and *Bacillus thuringiensis*.

26. The method of Claim 25 wherein the Bacillus host cell is *B.licheniformis*.

27. An enzymatic composition comprising a modified pullulanase.

28. The enzymatic composition of Claim 27 wherein the modified pullulanase has a deletion of amino acids from the amino terminus of up to about 100 amino acids.

30 29. The enzymatic composition of Claim 27 wherein the modified pullulanase has a deletion of amino acids from the amino terminus of up to about 200 amino acids.

35 30. The enzymatic composition of Claim 27 wherein the modified pullulanase has a deletion of amino acids from the amino terminus of up to about 300 amino acids.

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CONT

31. The composition of Claim 27 wherein the modified pullulanase has the amino acid sequence as shown in SEQ ID NO:2 beginning at amino acid residue 99, a glutamic acid.

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32. The composition of Claim 27 wherein the modified pullulanase has the amino acid sequence as shown in SEQ ID NO:2 beginning at amino acid residue 103, a glutamic acid.

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33. The composition of Claim 27 further comprising an enzyme selected from the group consisting of glucoamylase, alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, cyclomaltodextrin, glucotransferase, beta-glucanase, glucose isomerase, saccharifying enzymes, and/or enzymes which cleave glucosidic bonds.

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34. The composition of Claim 27 further comprising a glucoamylase.

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35. The composition of Claim 34 wherein the glucoamylase is obtainable from an Aspergillus strain.

36. The composition of Claim 35 wherein the Aspergillus strain includes Aspergillus niger, Aspergillus awamori and Aspergillus foetidus.

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37. The composition of Claim 27 wherein said composition is in a solid form.

38. The composition of Claim 27 wherein said composition is in a liquid form.

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39. The composition of Claim 27 comprising at least 60% modified pullulanase.

40. The composition of Claim 27 comprising at least 80% modified pullulanase.

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41. A process for the saccharification of starch, wherein said process allows for reduced concentrations of saccharification by-products, comprising the step of

contacting aqueous liquefied starch with an enzyme composition comprising modified pullulanase.

42. The process of Claim 41 wherein said modified pullulanase has a
5 deletion of up to about 100 amino acids, up to about 200 amino acids or up to about
300 amino acids from the amino terminus of pullulanase obtainable from a gram-negative or gram-positive microorganism.

10 43. The process of Claim 41 further comprising the steps of heating said
liquefied starch, and optionally recovering product.

15 44. The process of Claim 41 wherein said enzyme composition further
comprises glucoamylase.

20 45. The process of Claim 41 wherein said enzyme composition comprises at
least 80% modified pullulanase.

46. The process of Claim 41 wherein said contacting is at a pH of about less
than or equal to 7.0 and greater than or equal to 3.

25 47. The process of Claim 41 wherein the pH is about 4.2.

48. The process of Claim 41 wherein said heating is at a temperature range
of between 55 and 65 degrees C.

25 49. The process of Claim 41 wherein the temperature is about 60
degrees C.

30 50. B.licheniformis comprising nucleic acid encoding a modified pullulanase
wherein said B.licheniformis comprises a first gene encoding Carlsberg protease and
a second gene encoding endo Glu C protease, the first and/or second gene which
codes for the protease(s) having been altered such that the protease activity is
essentially eliminated.

35 51. B.licheniformis comprising nucleic acid encoding a mature pullulanase
wherein said B.licheniformis comprises a first gene encoding Carlsberg protease and

a second gene encoding endo Glu C protease, the first and/or second gene which codes for the protease(s) having been altered such that the protease activity is essentially eliminated.

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